water, the vaccine shall be rehydrated to 30 ml with mycoplasma medium. In the case of a cell line or a sample of primary cells, the inoculum shall consist of the resuspended cells. Control tests shall be established as provided in paragraph (d)(4) of this section.

(2) Inoculation of plate. Plate 0.1 ml of inoculum on an agar plate and make a short, continuous streak across the plate with a pipet. Tilt the plate to allow the inoculum to flow over the surface.

(3) Inoculation of flask of medium. Transfer 1 ml of the inoculum into a flask containing 100 ml mycoplasma medium and mix thoroughly. Incubate the flask at 33 to 37 °C for 14 days during which time, one of four agar plates shall be streaked with 0.1 ml of material from the incubating flask of inoculated medium on the 3d day, one on the 7th day, one on the 10th day, and one on the 14th day post-inoculation.

(4) Control tests shall be conducted simultaneously with the detection test using techniques provided in paragraphs (d)(2) and (3) of this section, except the inoculum for the positive control test shall be selected mycoplasma cultures and the negative control test shall be uninoculated medium from the same lot used in the detection test.

(5) All plates shall be incubated in a high humidity, 4–6 percent CO_2 atmosphere at 33 °to 37 °C for 10–14 days and examined with a stereoscopic microscope at 35x to 100x or with a regular microscope at 100x.

(e) Interpretation of test results.

(1) If growth appears on at least one of the plates in the positive control test and does not appear on any of the plates in the negative control test, the test is valid.

(2) If mycoplasma colonies are found on any of the plates inoculated with material being tested, the results are positive for mycoplasma contamination.

[38 FR 29887, Oct. 30, 1973, as amended at 41 FR 6752, Feb. 13, 1976; 41 FR 32882, Aug. 6, 1976]

§ 113.29 Determination of moisture content in desiccated biological products.

The moisture content shall be determined for each serial of desiccated product. the maximum moisture content for each product shall be established and an acceptable method used to determine the moisture content shall be described in an Outline of Production approved for filing by APHIS.

[54 FR 19352, May 5, 1989]

§ 113.30 Detection of Salmonella contamination.

The test for detection of Salmonella contamination provided in this section shall be conducted when such a test is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product.

(a) Samples shall be collected from the bulk suspension before bacteriostatic or bactericidal agents have been added. When tissue culture products are to be tested, 1 ml of tissue extract used as the source of cells or 1 ml of the minced tissue per se shall be tested.

(b) Five ml of the liquid vaccine suspension shall be used to inoculate each 100 ml of liquid broth medium (tryptose and either selenite F or tetrathionate). The inoculated media shall be incubated 18–24 hours at 35–37 °C.

(c) Transfers shall be made to either MacConkey agar or Salmonella-Shigella agar, incubated for 18–24 hours and examined.

(d) If no growth typical of Salmonella is noted, the plates shall be incubated an additional 18–24 hours and again examined.

(e) If suspicious colonies are observed, further subculture on suitable media shall be made for positive identification. If Salmonella is found, the bulk suspension is unsatisfactory.

[38 FR 29888, Oct. 30, 1973]

§ 113.31 Detection of avian lymphoid leukosis.

The complement-fixation test for detection of avian lymphoid leukosis provided in this section shall be conducted on all biological products containing virus which has been propagated in substrates of chicken origin: *Provided*, An inactivated viral product shall be exempt from this requirement if the licensee can demonstrate to Animal and Plant Health Inspection Service that